Quantitative assessment of the bacterial rhizosphere flora of *Pinus contorta* var. *latifolia*[1]

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The bacterial flora associated with root systems of young and mature lodgepole pine was investigated by sampling forest-grown trees. Counts were performed and expressed on a surface-area basis to give a more realistic comparison of organism density or activity within the control soil, rhizosphere soil, and rhizoplane. On this basis, densities increased by an order of 10^4- to 10^6-fold from control soil to rhizoplane, with the degree of stimulation being inversely related to root radius.

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La flore bactérienne associée aux systèmes racinaires du Pin de Murray juvénile ou à maturité a été étudiée par échantillonnage d'arbres en forêt. Les comptes bactériens furent établis et exprimés sur une base de superficie pour fins de comparaison plus réaliste de la densité ou de l'activité des organismes du sol témoin, le sol de la rhizosphère et du rhizoplan. Sur cette base, les densités augmentent de l'ordre de 10^4 à 10^6 fois, du sol témoin au rhizoplan, avec un degré de stimulation inversement proportionnel au rayon de la racine.

Introduction

The rhizosphere microflora of agricultural crops has been intensively investigated and a quantitative stimulation universally reported (Katznelson 1961). In contrast, the work with forest plant species under field conditions has been limited and is restricted to observations on Douglas fir (Neal *et al.* 1968), alder (Neal *et al.* 1968), birch and spruce (Runov and Zhdannikova 1960), and oak, larch, and fir (Samstevich 1956). A quantitative stimulation has not always been reported for these plants (Samtsevich 1956).

The standard method of comparing activities among plant root regions (control soil, rhizosphere soil, and rhizoplane) is to compare bacterial counts per gram oven-dried material (i.e., organism density) for these regions; the organism density serving as an indicator of microbial activity. The comparison of control soil and rhizoplane counts on this basis is unrealistic because the soil organisms are spread throughout the porous soil matrix, whereas the rhizoplane organisms are located on and in the surface layers of the root cylinder. Harper (1950) states, "Ideally, the expression of the rhizosphere effect should take into account the amount of root present and perhaps the area of root surface."

The primary objective of this study was to examine the bacterial flora associated with root systems of *Pinus contorta* var. *latifolia* as found under natural field conditions. As surface area has not been used as a basis for comparing activities of control soil, rhizosphere soil, and the rhizoplane areas, all data were calculated both on a weight and on a surface-area basis.

Materials and Methods

Sampling

Young trees, 5 to 6 years old and about 12 cm high, were collected and transported in an ice chest to the laboratory. The trees were growing in a sandy loam soil on a cleared power line right-of-way from which all of the surface organic horizon had been scraped away. Only trees which were greater than 20 cm away from all competing vegetation were sampled. The method used to separate rhizosphere and rhizoplane material on these samples was that of Louw and Webley (1959). To obtain the rhizosphere material, the root systems and adhering

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soil were placed in sterile water and gently rotated to free adhering soil before removing roots. The rhizosphere and control soils were sampled after 5 min of shaking at 600 oscillations per minute (2.5 cm orbit). The rhizoplane flora contained on the roots was obtained by transferring the root to a sterile flask containing 4 g of 1-mm glass beads per 10 ml of water. This was then shaken as above for 20 min. Oven-dry weight of soil or roots in each sample was determined by drying to constant weight at 100 °C.

Larger trees, growing on a loamy sand soil, were selected for sampling from a stand of lodgepole pine (*Pinus contorta* var. *latifolia*) that averaged 27 years of age and 6 m in height. Holes were dug so that one surface of the root was exposed, marked in 30-cm sections, and traced as far as possible through the soil. The rhizosphere was sampled by scraping the soil immediately adjacent to the root in each 30-cm section into a sterile 18-mm tube. The control sample was collected 15 cm from the root by scraping freshly exposed soil into another 18-mm tube. The root system was then cut into the marked sections and transported to the laboratory in an ice chest. Rhizoplane samples from the larger roots were prepared by peeling measured bark fragments from the different 30-cm root sections, placing them in a flask, and treating them as described for the young tree rhizoplane.

Microbiological

Aliquots from samples were serially diluted in sterile distilled water. Previous tests indicated that counts after dilution in water were not significantly different from those obtained when phosphate buffer was used for dilution and were significantly greater than those obtained with Ringers solution and soil extract. Five replicates from each of three dilutions were inoculated by spreading 0.1 ml of inoculum on medium of the following composition: glucose, yeast extract, and K_2HPO_4, 0.1%; $(NH_4)_2SO_4$, 0.5%; $MgSO_4 \cdot 7H_2O$, 0.02%; $CaCl_2$ and NaCl, 0.01%; agar, 1.5%; trace of iron and actidione at 80 ng/ml. Colonies were counted after 18 days of incubation at 20 °C.

Root and Soil Surface Areas

Soil sample surface area was determined using the ethylene glycol monoethyl ether (EGME) technique (Heilman *et al.* 1965). Samples were saturated with calcium and ground to pass a 60-mesh sieve before EGME treatment.

Surface area of roots was determined in either of two ways. For small roots, the total length of root systems was measured after carefully washing and blotting dry. These root systems were then cut into segments, placed in a wire-mesh basket, and the root volume determined by displacement in ethanol (Baver 1956). The volume of the root system is the difference between root weight in air and root weight in ethanol multiplied by the inverse of the specific gravity of ethanol. By using the root length and volume, the average root radius was calculated ($V = \pi r^2 h$), as well as surface area ($SA = 2\pi rh$). After determination of root system volume, the root segments were oven-dried at 100 °C and weighed to allow for the calculation of an average surface area per gram of dry root system. With larger root systems, bark sections were squared and measured before their use as rhizoplane samples. These measurements allowed the direct calculation of the total surface area of the fragments.

TABLE 1

Total bacterial and streptomycete counts from control soil (S) at the mature tree sampling site

Sample depth, cm	Soil SA,* m^2/g	Average nos./g	Nos./100 cm^2
15–45	14.9	67	447
45–75	14.9	119	798
75–105	14.9	84	561
105–135	14.9	34	225
135–165	14.9	3	19

*Soil surface area.

Results

Mature Tree Studies

Table 1 indicates the average counts for the control soil from the mature tree sampling site. The soil on this site is a Degraded Dystric Brunisol (Canada Soil Survey Committee 1974) developed on a loamy sand parent material with a pH of 4.3 in the B horizon. Control soil counts are similar to those found on other Degraded Dystric Brunisolic soils developed on coarse-textured parent material and supporting lodgepole pine (Dangerfield, unpublished data).

Tables 2 and 3 summarize the average counts for rhizosphere soil and rhizoplane areas of the mature tree sampling. A distinct rhizosphere effect is not noted until root diameter decreases to about 2.5 mm. There is, however, a marked rhizoplane effect at all root diameters, which varies inversely with root diameter.

Young Tree Studies

Because of the damage to seedling roots that resulted from shaking with glass beads, determination of root surface area on samples used for bacterial counts was impossible. This problem was circumvented by establishing an average value for the relationship between oven-dry weight of roots and root surface area. To develop this relationship, portions of the root systems of 13 seedlings similar in height and weight to the four sampled for bacterial counts were characterized. Average root radius, root weight, and root surface areas determined by suspending in ethanol and in water plus Tween 80 are summarized in Table 4. A straight line

TABLE 2

Total bacterial and streptomycete counts from rhizosphere soil (R) at the mature tree sampling site

Sample depth, cm	Root diam, mm	Soil SA,* m^2/g	Average nos./g	Nos./100 cm^2	R/S†
15–45	41	14.9	85	571	1.3
45–75	17	14.9	176	1180	1.5
75–105	2.52	14.9	589	3950	7.2
105–135	2.26	14.9	392	2630	10.2

*Soil surface area.
†R/S ratio of rhizosphere soil numbers/100 cm^2 (Table 2) to control soil numbers/100 cm^2 (Table 1).

TABLE 3

Total bacterial and streptomycete counts from rhizoplane (Rh) at the mature tree sampling site

Sample depth, cm	Root diam, mm	Root SA,* cm^2/g	Average nos./g	Nos./100 cm^2	Rh/S† $\times 10^4$
15–45	41	23.2	188	811	1.8
45–75	17	29.5	648	2235	2.8
	1.6	36.9	7720	20900	26
	1.08	50.2	2860	5720	7.2
75–105	2.52	29.9	3840	12800	23
105–135	2.26	31.4	1210	3870	17
135–165	1.44	43.3	2020	4660	25

*Root surface area.
†Rh/S ratio of rhizoplane numbers/100 cm^2 (Table 3) to control soil numbers/100 cm^2 (Table 1).

TABLE 4

Surface area of roots sampled from lodgepole pine seedlings

Seedling No.	Average root radius, cm	Surface area, cm^2		Root oven-dry wt.	SA/g*
		Ethanol	H_2O plus Tween 80		
1	0.057	24.2	24.1	0.253	96
2	0.079	10.4	10.6	0.172	61
3	0.036	29.2	30.7	0.288	102
4	0.035	34.0	33.6	0.295	115
5	0.041	39.0	40.8	0.369	106
6	0.039	21.1	20.4	0.173	122
7	0.032	26.5	25.8	0.188	141
8	0.035	14.4	14.5	0.115	126
9	0.033	23.1	ND†	0.30	77
10	0.039	40.5	ND	0.46	88
11	0.027	26.0	ND	0.25	104
12	0.059	48.0	ND	1.0	48
13	0.076	20.8	ND	0.49	42

*Surface area expressed in square centimeters.
†Not determined. Average root surface area from all seedlings is 95.5 cm^2/g. Average root surface area from seedlings with root radius less than 0.05 cm is 109 cm^2/g.

TABLE 5

Bacterial counts from four young trees

Tree sample no.	Soil or root SA* $\times 100\ cm^2/g$	Total counts/g oven-dry wt. $\times 10^4$	SD $\times 10^4$	Total counts per $100\ cm^2$	$\frac{R/S\dagger}{A}$	or $\frac{Rh/S\ddagger}{B}$
Control soil (S)						
1	7850	14	±2.5	18.1		
2	7380	15	±1.9	19.8		
3	9430	51	±9.6	54.5		
4	9020	39	±1.7	42.9		
Rhizosphere soil (R)						
1	7850	420	±29	538	30	30
2	7380	890	±63	1206	61	61
3	9430	680	±39	718	13	13
4	9020	730	±82	815	19	19
Rhizoplane (Rh)						
1	1.09	5200	±490	48×10^6	367	27×10^5
2	1.09	10600	±930	97×10^6	726	49×10^5
3	1.09	5600	±730	51×10^6	108	9×10^5
4	1.09	10900	±1200	100×10^6	282	23×10^5

*Soil or root surface area.
†Comparison based on total counts per gram of sample.
‡Comparison based on total counts/100 cm^2 of surface.

relationship can be obtained from these data by plotting $\log_{10}$ surface area per gram against root radius. The average value selected to represent the relationship between oven-dry weight of roots and root surface area (109 cm^2/g) was obtained by averaging the surface area of all root systems with an average radius of 0.05 cm or less (Table 4).

Table 5 indicates the counts from control soil, rhizosphere soil, and rhizoplane area for the four seedlings sampled. The rhizosphere effect is again demonstrated as is the large rhizoplane activity. The values for rhizoplane densities are larger than for the older trees and expand the trend of increasing rhizoplane activity with decreasing root radius established for the larger trees.

Discussion

The expected rhizosphere effect is demonstrated and similar in magnitude to values reported for agricultural crops (Alexander 1961) and greater than the values reported by Timonin (1966) for lodgepole pine reared under artificial conditions. The values reported are also larger than those noted for yellow birch (Ivarson and Katznelson 1960), yellow poplar (Shipman 1957), and red alder, and Douglas fir (Neal *et al.* 1968). The large rhizosphere effect may be a product of the poor nutritional quality of the soil, since the rhizosphere effect will be more strongly expressed in poor soils (Runov and Zhdannikova 1960). As expected, the greatest numbers of organisms were found in the rhizoplane but the magnitude of this increase in numbers was unexpected. This unexpected increase is not an artifact resulting from the choice of an inappropriate base of comparison (i.e., surface area).

Data in the literature support the validity of the use of surface area as the basis for comparing control soil and rhizoplane activities. Stotzky (1966) stressed the possible importance of surface area in stimulating bacterial metabolism. This idea has been substantiated by subsequent information appearing in the literature. For example, Parr *et al.* (1967) indicated that under certain conditions one factor limiting bacterial activity was competition for available linear surface, and Marshall (1971) clearly showed that soil bacteria live predominately adsorbed to soil surfaces. These observations plus those reported in this paper indicate that it is more realistic to express soil bacterial numbers on a surface area basis than on a weight basis.

The root is a tubular structure and the bacteria present can be considered to be located only on the cylindrical surface of the structure. Under-

standably, there are errors and limitations in the root surface area calculations, but these can be minimized. The root surface area for the larger trees was determined by measuring the dimensions of root sections and should thus be subject to limited error. The Rh/S[2] values for the mature tree sampling are not markedly different from the Rh/S values for the younger trees. It is reasonable to expect increased root surface activity with decreasing root size as the smaller younger roots are the sites of active growth and thus the region of maximum root exudation.

Recently, Rovira *et al.* (1974) examined microscopically the distribution of rhizoplane organisms on the roots of four grasses and four dicotyledons and found the average bacterial cover to range from 4 – 10%. Assuming an average bacterial size of 1 μm^2 (Rovira *et al.* 1974) and calculating with data presented in Table 3, the bacterial cover on the roots examined in this study range from 0.2 to 2.1%. Our values are in agreement with those of Rovira *et al.* (1974). We suggest that the use of surface area as a basis for comparing root-associated microbial activities is a more realistic measure of activity than comparisons on a weight basis.

These results indicate the magnitude of microbial activity on the root surface and emphasizes the importance of concentrating future studies of root-associated microorganisms on the rhizoplane microflora.

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[2]Rh, rhizoplane. S, control soil.